01-05-01
526 Rec'd PCT/PTO 05 JAN 2001

FORM PTO-1390 (REV 10-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		204,940
		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/744599
INTERNATIONAL APPLICATION NO. PCT/EP99/04604	INTERNATIONAL FILING DATE July 2, 1999	PRIORITY DATE CLAIMED July 6, 1998

TITLE OF INVENTION BIOCOMPATIBLE AND BIODEGRADABLE COMPOSITIONS CONTAINING HYALURONIC AND THE DERIVATIVES THEREOF FOR THE TREATMENT OF ULCERS IN THE DIGESTIVE APPARATUS

APPLICANT(S) FOR DO/EO/US
Lanfranco CALLEGARO, Luigi AMBROSIO AND Annaclaudia ESPOSITO

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. [X] The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. [ ] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [ ] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). - unsigned
10. [ ] An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 16 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information: PCT Notification of Transmittal with PCT International Preliminary Examination Report; Forms: PCT/IB/308 and PCT/IB/332

534 Rec'd PCT/PTO 05 JAN2001

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 09/744333	INTERNATIONAL APPLICATION NO. PCT/EP99/04604	ATTORNEY'S DOCKET NUMBER 204,940

	CALCULATIONS PTO USE ONLY	
17. ☒ The following fees are submitted:		
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**	$ 860.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	24 - 20 =	4	X **$18.00**	$ 72.00	
Independent claims	- 3 =		X **$80.00**	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	$	
TOTAL OF ABOVE CALCULATIONS =				$ 72.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$	
SUBTOTAL =				$ 932.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 932.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ 932.00	
				Amount to be refunded:	$
				charged:	$

a. ☒ A check in the amount of $ 932.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 01-0035. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

ABELMAN FRAYNE & SCHWAB
Attorneys at Law
150 East 42nd Street
New York, NY 10017
(212) 949-9022

Jan.5, 2001

SIGNATURE

Jay S. Cinamon

NAME

24,156

REGISTRATION NUMBER

PATENT DOCKET 204,940

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	CALLEGARO ET AL.	EXAMINER:
SERIAL NO.:	Not Yet Assigned	ART UNIT.:
FILED:	Herewith	
TITLE:	BIOCOMPATIBLE AND BIODEGRADABLE COMPOSITIONS CONTAINING HYALURONIC ACID AND THE DERIVATIVES THEREOF FOR THE TREATMENT OF ULCERS IN THE DIGESTIVE APPARATUS	

DATE: January 5, 2001

PRELIMINARY AMENDMENT

Hon. Commissioner of
Patents and Trademarks
Washington, D.C. 20231

SIR:

STATEMENT OF FILING BY EXPRESS MAIL 37 C.F.R. § 1.10
This correspondence is being deposited with the United States Postal Service on January 5, 2001 in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EK 908123671 US addressed to the Honorable Commissioner for Patents, Washington, D.C. 20231.

Please amend the application filed on even date herewith prior to proceeding with its examination.

IN THE CLAIMS

8. (Amended) The use according to claim 1, wherein the hyaluronic acid derivatives are amides wherein a deacylated amino group of hyaluronic acid or of a [derivative thereof as defined in claims 2-6,] hyaluronic acid ester wherein part or all of the carboxy functions are esterified with an alcohol selected from the group consisting of aliphatic, aromatic, arylaliphatic, cycloaliphatic and heterocyclic series, is reacted with an acid [chosen] selected from the group consisting of [the] aliphatic, aromatic, arylaliphatic [or] and cyclo aliphatic acids, that optionally can be a pharmaceutically active substance.

Claim 12, lines 7-8, delete "claims 2-8", insert --claim 2--.

Please add new claims 15 through 24.

--15. The use according to claim 1, wherein the hyaluronic acid derivatives are amides wherein a deacylated amino group of hyaluronic acid or of a cross-linked ester of hyaluronic acid wherein part or all of the carboxy groups are esterified with the alcoholic functions of the same polysaccharide chain or other chains, is reacted with an acid chosen from the group consisting of aliphatic, aromatic, arylaliphatic and cycloaliphatic acids, that optionally can be a pharmaceutically active substance.--

--16. The use according to claim 1, wherein the hyaluronic acid derivatives are amides wherein a deacylated amino group of hyaluronic acid or of a cross-linked compound of hyaluronic acid wherein part or all of the carboxy groups are esterified with polyalcohols of the

aliphatic, aromatic, arylaliphatic, cycloaliphatic, and heterocyclic series, generating cross-linking by means of spacer chains, is reacted with an acid selected from the group consisting of the aliphatic, aromatic, arylaliphatic and cycloaliphatic acids, that optionally can be a pharmaceutically active substance.--

--17. The use according to claim 1, wherein the hyaluronic acid derivatives are amides wherein a deacylated amino group of hyaluronic acid or of a hemiester of succinic acid or heavy metal salts of the hemiester of succinic acid with hyaluronic acid or partial or total esters of hyaluronic acid, is reacted with an acid selected from the group consisting of aliphatic, aromatic, arylaliphatic and cycloaliphatic acids, that optionally can be a pharmaceutically active substance.--

--18. The use according to claim 1, wherein the hyaluronic acid derivatives are amides wherein a deacylated amino group of hyaluronic acid or of a O-sulfated or N-sulfated hyaluronic acid derivative, is reacted with an acid selected from the group consisting of aliphatic, aromatic, arylaliphatic and cycloaliphatic acids that optionally can be a pharmaceutically active substance.--

--19. A biological material comprising:

a) intestinal cells optionally together with fibroblast, mesenchimal cells, mature cells and/or epithelial cells;

b) a matrix comprising at least one hyaluronic acid derivative as defined in claim 3.--

--20. A biological material comprising:

a) intestinal cells optionally together with fibroblasts, mesenchimal cells, mature cells and/or epithelial cells;

b) a matrix comprising at least one hyaluronic acid derivative as defined in claim 4.--

--21. A biological material comprising:

a) intestinal cells optionally together with fibroblasts, mesenchimal cells, mature cells and/or epithelial cells;

b) a matrix comprising at least one hyaluronic acid derivative as defined in claim 5.--

--22. A biological material comprising:

a) intestinal cells optionally together with fibroblasts, mesenchimal cells, mature cells and/or epithelial cells;

b) a matrix comprising at least one hyaluronic acid derivative as defined in claim 6.--

--23. A biological material comprising:

a) intestinal cells optionally together with fibroblasts, mesenchimal cells, mature cells and/or epithelial cells;

b) a matrix comprising at least one hyaluronic acid derivative as defined in claim 7.--

--24. A biological material comprising:

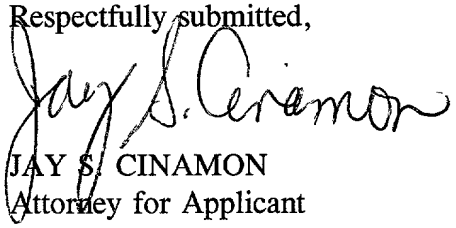
a) intestinal cells optionally together with fibroblasts, mesenchimal cells, mature cells and/or epithelial cells;

b) a matrix comprising at least one hyaluronic acid derivative as defined in claim 8.--

REMARKS

It is respectfully requested that the examination of claims 1-24, inclusive, of this National Phase Application proceed on the basis of the amendatory action taken herein, and that this amendment be entered prior to calculating the filing fee and according the application a filing date.

Respectfully submitted,



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BIOCOMPATIBLE AND BIODEGRADABLE COMPOSITIONS CONTAINING HYALURONIC ACID AND THE DERIVATIVES THEREOF FOR THE TREATMENT OF ULCERS IN THE DIGESTIVE APPARATUS

Field of the invention

The present invention concerns the use of hyaluronic acid or the derivatives thereof for the preparation of a composition to treat ulcers, lesions and diverticula of the digestive and gastrointestinal apparatus.

Said compositions can optionally comprise pharmacologically or biologically active substances. Said compositions comprising cells can be used for the reconstruction of the wall of the digestive apparatus

Technical Background

Therapy for gastric ulcers has long depended on H2 receptor antagonists such as cimetidine and ranitidine, able to inhibit gastric secretion.

Recently, drugs such as omeprazole which reduce the secretion of gastric acid by a specific mechanism of inhibition of the proton pump at a parietal cell level have been used.

Said drugs are often associated with antibiotics such as amoxacillin, tetracycline, metronidazole and claritromycin that are efficacious against Helicobacter pylori, the gram-negative micro-organism held to be responsible for the recurrence of peptic ulcers.

Surgery is resorted to only in the case of gastric ulcers, suspected to be malignant, or in cases of peptic ulcers complicated by stenosis impairing the passage of food. Cases of perforation also urgently require surgery to close the lesion and avoid potentially fatal consequences such as peritonitis.

Another pathology affecting the digestive system is diverticulitis of the oesophagus.

Diverticula of the digestive tract, particularly the oesophagus, are circumscribed, funnel-shaped or saccate extroversions, that may involve all the layers, or just the mucosa and submucosa of the gut wall.

Food residues stagnating in a diverticulum cause its inflammation, and may also find their way into the respiratory system as a result of coughing or regurgitation,

causing bronchitis, bronchial pneumonia and pulmonary abscesses ab ingestis. Complications such as esophago-bronchial fistulae, oesophagitis, haemorrhages, malignant neoplasia of the diverticulum may also occur.

Treatment of the pathology, must include a suitable diet and the use of antispastic and prokinetic drugs, while more serious cases may require surgery.

Ulcers in the digestive tract may be caused not only by pathologies but also by external trauma, by swallowing sharp foreign bodies or caustic substances.

In the latter case, surgery may prove useless and the affected part may irretrievably lose its functions of absorption, secretion and peristalsis.

The role of hyaluronic acid, a natural polysaccharide, in the process of tissue repair has long been known (Weigel, P. H. et al.: "A model for the role of hyaluronic acid and fibrin in the early events during the inflammatory response and wound healing", *J. Theor. Biol.*, 119: 219, 1986), especially in the early stages of granulation, as it stabilises the coagulation matrix and controls its degradation, favouring the recruitment of inflammatory cells such as polymorphonucleate leukocytes and monocytes, mesenchymal cells such as fibroblasts and endothelial cells, and, lastly, orienting subsequent migration of the epithelial cells.

For the above reasons, hyaluronic acid is widely used in pharmaceutical formulations in the form of creams, sprays and gauzes (Connettivina®) able to accelerate the healing of sores, wounds and burns (EP 0138572).

Moreover, hyaluronic acid derivatives (EP 0216453 B1) are known to be used as scaffolds for the culture of cells such as fibroblasts, keratinocytes, bone marrow stem cells (PCT WO 97/18842) to prepare grafts of bone tissue, cartilage and skin.

Lastly, there are known pharmaceutical preparations in the form of tablets or granules containing esters of acidic polysaccharides with choline (EP 0605478) with antiulcer properties at a gastric level due to their ability to form gels and protect the mucosa.

Although hyaluronic acid and its derivatives are used topically, their application in the digestive and gastrointestinal systems is as yet unknown.

It has now been discovered, surprisingly, that compositions based on hyaluronic

acid or its derivatives, optionally in association with growth factors or cell cultures, suitable for oral or endoscopic administration, can be used effectively in the treatment of lesions or ulcers in the digestive and gastrointestinal systems.

These are able to spread and adhere to the inner walls of the digestive apparatus, protecting the mucosa and stimulating tissue regeneration, possibly exercising an antibacterial activity if physically or chemically associated with substances having such properties.

In more serious cases, for example where there is loss or degeneration of a large area of tissue or perforation of the wall of the digestive apparatus, surgery can be performed and it is possible to reconstruct the injured part by grafting cell cultures grown on scaffolds constituted by hyaluronic acid derivatives.

Summary of the invention

The Applicant has unexpectedly found that hyaluronic acid and derivatives thereof may be advantageously used in the treatment of ulcers, lesions and diverticula of the digestive and gastrointestinal apparatus.

The present invention therefore relates to the use of hyaluronic acid or a derivative thereof for the preparation of pharmaceutical compositions for the treatment of the above mentioned diseases.

Brief description of the drawings

Figure 1 shows electron microscope image (Mag: 14.97 K X) on the 38th day of culture grown on Petri dishes;

Figure 2 shows electron microscope image (Mag: 17.50 K X) on the 38th day of culture grown on transwells;

Figure 3 shows electron microscope image (Mag: 17.50 K X) on the 38th day of culture grown on Laserskin® (bidimensional matrix comprising hyaluronic acid esters);

Figure 4 shows electron microscope image (Mag: 15.02 K X) on the 38th day of culture grown on Hyaff11 3D (three-dimensional matrix comprising hyaluronic acid esters);

Figure 4a shows electron microscope image (Mag: 898 X) on the 38th day of culture grown on Hyaff11 3D (three-dimensional matrix comprising hyaluronic acid

esters);

Figure 5 shows electron microscope image (Mag: 15.02 K X) on the 38th day of culture grown on polyurethane;

Figure 6 shows diagrams with in ordinates ALP (alkaline phosphates) Activity (mU/mg of proteins) and in abscissae days of culture.

Detailed description of the invention

Of the hyaluronic acid derivatives that can be used according to the present invention the following are to be preferred:

- hyaluronic acid esters wherein part or all of the carboxy functions are esterified with alcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series (EP 0216453 B1 entirely incorporated by reference);
- autocross-linked esters of hyaluronic acid wherein part or all of the carboxy groups are esterified with the alcoholic functions of the same polysaccharide chain or other chains (EP 0341745 B1 entirely incorporated by reference);
- cross-linked hyaluronic acid compounds wherein part or all of the carboxy groups are esterified with polyalcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic or heterocyclic series, generating cross-linking by means of spacer chains (EP 0265116 B1 entirely incorporated by reference);
- hemiesters of succinic acid or the heavy metal salts of the hemiester of succinic acid with hyaluronic acid or with partial or total esters of hyaluronic acid (WO 96/357207 entirely incorporated by reference);
- O-sulphated derivatives (WO 95/25751 entirely incorporated by reference) or N-sulphated derivatives (PCT/EP98/01973 entirely incorporated by reference);
-amidic derivatives of hyaluronic acid or of the compounds listed above obtained by reaction of a primary or secondary amine of the aliphatic, aromatic, arylaliphatic, cycloaliphatic or heterocyclic series, that can optionally be a pharmaceutically active substance, with a free carboxylic group of hyaluronic acid or a derivative thereof; or by reaction of an acid of the aliphatic, aromatic, arylaliphatic or cycloaliphatic series, that can optionally be a pharmaceutically active substance, with a deacylated amino group of hyaluronic acid or a derivative thereof.

The compositions according to the present invention may also contain pharmacologically or biologically active substances such as antibiotics, in particular antibiotics active against Helicobacter pylori, growth factors, antimicotics, antimicrobials and antiviral agents. Compositions containing antibiotics active against Helicobacter pylori are for example in the form of mixtures or salts, or covalently bound with the aforesaid hyaluronic acid derivatives; heavy metal salts such as zinc and cobalt, salts of the hemiester of succinic acid or hyaluronic acid or with partial or total esters of hyaluronic acid.

The hyaluronic acid or hyaluronic acid derivatives used according to the present invention are preferably in the form of gels, guide channels, sponges, non-woven fabric, threads, continuous or perforated membranes, microspheres, nanospheres, gauzes or associations of the same.

In particular microspheres and nanospheres can be processed in the form of tablets, capsules, suspensions or solutions.

Therefore a further subject of the present invention relates to oral compositions suitable to be absorbed by the gastrointestinal mucose containing a hyaluronic acid derivative as the active ingredient for the treatment of ulcers, lesions and diverticula of the digestive and gastrointestinal apparatus.

The aforementioned active substances different from hyaluronic acid and the derivatives thereof may also be vehicled in hyaluronic acid and the derivatives thereof in the form of microspheres and nanospheres as disclosed respectively in EP A 517565 and WO 96/29998

Bidimensional or three dimensional matrix containing a hyaluronic acid derivative, may be used as support for cellular growth for the preparation of biological material containing suitable cell cultures for regenerating the walls or filling diverticula in the digestive apparatus. Said cells can be mature intestinal cells, mesenchymal cells, fibroblasts, epithelial cells or mixture thereof.

These biological materials for example may contain intestinal cells useful in the reconstruction of injured digestive apparatus. These biological materials are implanted onto the lesion site by surgical methods.

EXAMPLE

Growth of epithelial cells on scaffolds made of benzyl esters of hyaluronic acid

Intestinal cells were seeded onto scaffolds made of the total benzyl ester of hyaluronic acid in the form of a perforated membrane and non-woven fabric, in order to test their biocompatibility, and their morphological and biochemical responses were observed.

The cells belonged to the $CaCO_2$ cell line (derived from human colon carcinoma) that differentiate spontaneously into enterocytes typical of the mature intestinal epithelium.

The cells were used at passage 98. They were seeded at a density of about 9 x $10^3/cm^2$ in DMEM 4.5 g of glucose/L containing 20% FBS penicillin/streptomycin, fungizone and non-essential amino acids (1%) in a humidified atmosphere with 95% CO_2. The culture medium was changed every 48 hours. Other cells were seeded on Petri dishes and Transwell wells with polycarbonate membranes in the same culture conditions and served as controls.

Polyurethane (chronoflex_TM), a material for biomedical purposes, was used as negative control. On the 3rd, 15th, 20th and 40th days of culture, the cells were prepared for observation user scanning electron microscope (SEM) and for assessment of the total proteins and the activity of alkaline phosphates (ALP) according to the following methods: SEM fixing in 2.5% glutaraldehyde in phosphate buffer (PBS) pH 7.4. Osmium tetroxide, 1% in PBS, dehydration in ethanol and increasing concentrations of up to 100% and dehydration with a Critical Point drier. The cells were then metalized with gold and observed by SEM.

ALP activity: the cells were harvested by scraping in a lysis buffer 2mM Tris-HCl 50 mM mannitol pH 7.2 (1 ml final volume) (with the exception of those seeded on Hyaff 3D) and sonicated in ice. ALP activity of the cellular lysates was determined by spectrophotometry by hydrolysis of the p-nitrophenylphosphate using a Boehringer kit. The total proteins were determined by Lowry's method. The activity present in the cells grown on a scaffold in the form of a non-woven fabric was determined in lysates obtained by sonicating the membrane containing the cells *in toto*.

Morphological differentiation was assessed on the basis of the presence of microvilli on the upper surface of the cells, while the biochemical differentiation was assessed on the basis of the increase of ALP activity (see results in Figure 6). Both were considered as biocompatibility parameters.

Figures 1, 2, 3, 4, 4a and 5 show electron microscope images of the cells on the 38th day of culture, grown on Petri dishes, transwells, membranes of hyaluronic acid (Laserskin®), hyaluronic acid matrices (Hyaff11 3D) and polyurethane membranes respectively. As can be seen, the cells grown on Laserskin and Transwell show marked differentiation due to the appearance of numerous microvilli on their surfaces, whereas those grown on Petri dishes show fewer, less well developed microvilli. The cell grown on the scaffold (in the form of a non-woven fabric) and Chronoflex do not show any formation of microvilli, while those grown on Chronoflex alone present extroversion indicative of cell suffering.

AR/34

CLAIMS

1. Use of a matrix comprising at least one hyaluronic acid or a derivative thereof, as a support for cellular growth for the preparation of biological material for the treatment of ulcers, lesions and diverticula of the digestive and gastrointestinal apparatus.

2. The use according to claim 1, wherein the hyaluronic acid derivatives are hyaluronic acid esters wherein part or all of the carboxy functions are esterified with alcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series.

3. The use according to claim 1, wherein the hyaluronic acid derivatives are the cross-linked esters of hyaluronic acid wherein part or all of the carboxy groups are esterified with the alcoholic functions of the same polysaccharide chain or other chains.

4. The use according to claim 1, wherein the hyaluronic acid derivatives are the cross-linked compounds of hyaluronic acid wherein part or all of the carboxy groups are esterified with polyalcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, generating cross-linking by means of spacer chains.

5. The use according to claim 1, wherein the hyaluronic acid derivatives are hemiesters of succinic acid or heavy metal salts of the hemiester of succinic acid with hyaluronic acid or partial or total esters of hyaluronic acid.

6. The use according to claim 1, wherein the hyaluronic acid derivatives are O-sulphated or N-sulphated hyaluronic acid derivatives.

7. The use according to claim 1, wherein the hyaluronic acid derivatives are hyaluronic acid amides wherein part or all the free carboxylic groups of hyaluronic acid are reacted with a primary or a secondary amine chosen from the group consisting of the aliphatic, aromatic, arylaliphatic, cycloaliphatic or heterocyclic amine, that can optionally be a pharmaceutically active substance.

8. The use according to claim 1, wherein the hyaluronic acid derivatives are amide wherein a deacylated amino group of hyaluronic acid or of a derivative thereof as defined in claims 2-6, is reacted with an acid chosen from the group consisting of the aliphatic , aromatic, arylaliphatic or cycloaliphatic acid, that can optionally be a

pharmaceutically active substance.

9. The use according to claim 1, wherein the said matrix is in the form of a non woven fabric.

10. The use according to claim 1, wherein the said matrix is in the form of a perforated membrane.

11. The use according to claim 1, wherein the cells are chosen from the group consisting of mature cells, mesenchimal cells, fibroblasts, epithelial cells and mixtures thereof.

12. A biological material comprising:

a) intestinal cells optionally together with fibroblasts, mesenchimal cells, mature cells and/or epithelial cells;

b) a matrix comprising at least one hyaluronic acid derivative as defined in claims 2-8.

13 The biological material according to claim 12, wherein said matrix is in the form of a non woven tissue.

14. The biological material according to claim 12, wherein said matrix is in the form of a perforated membrane.

FIGURE 1

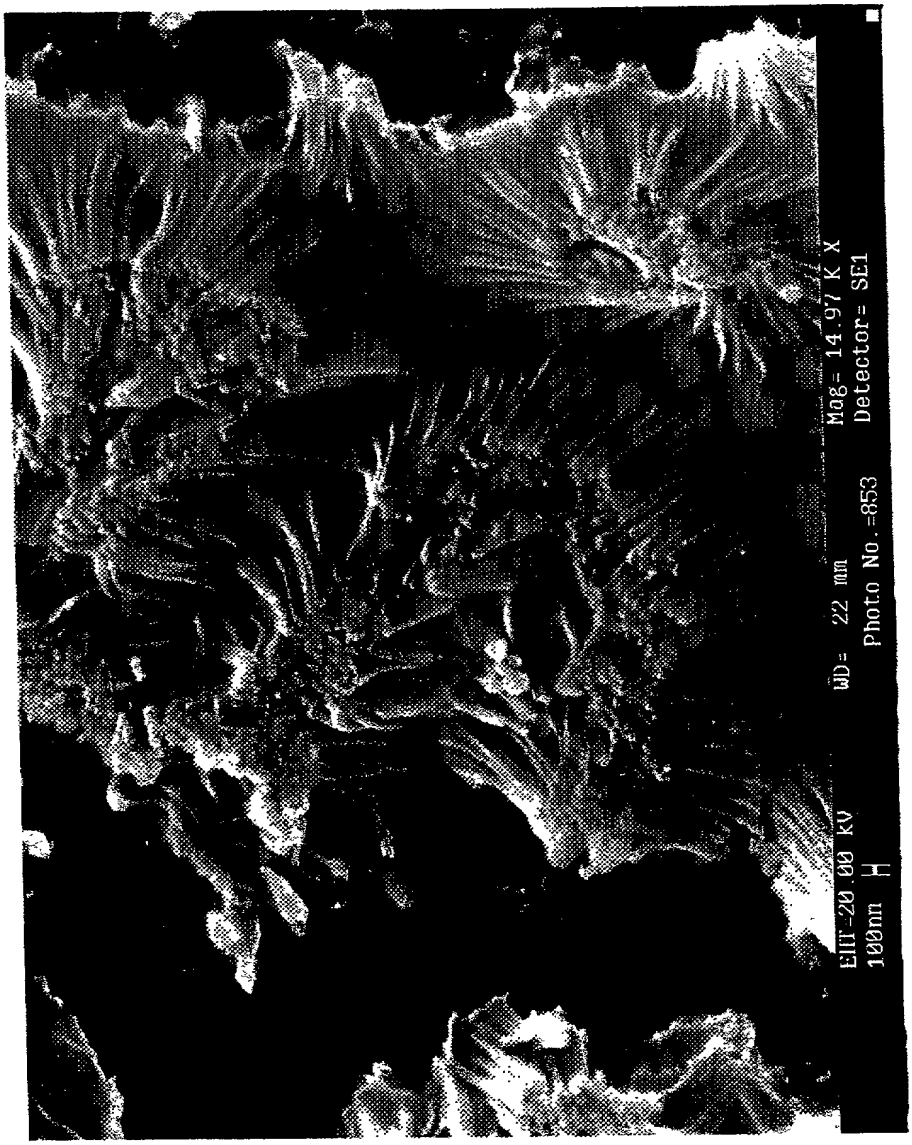
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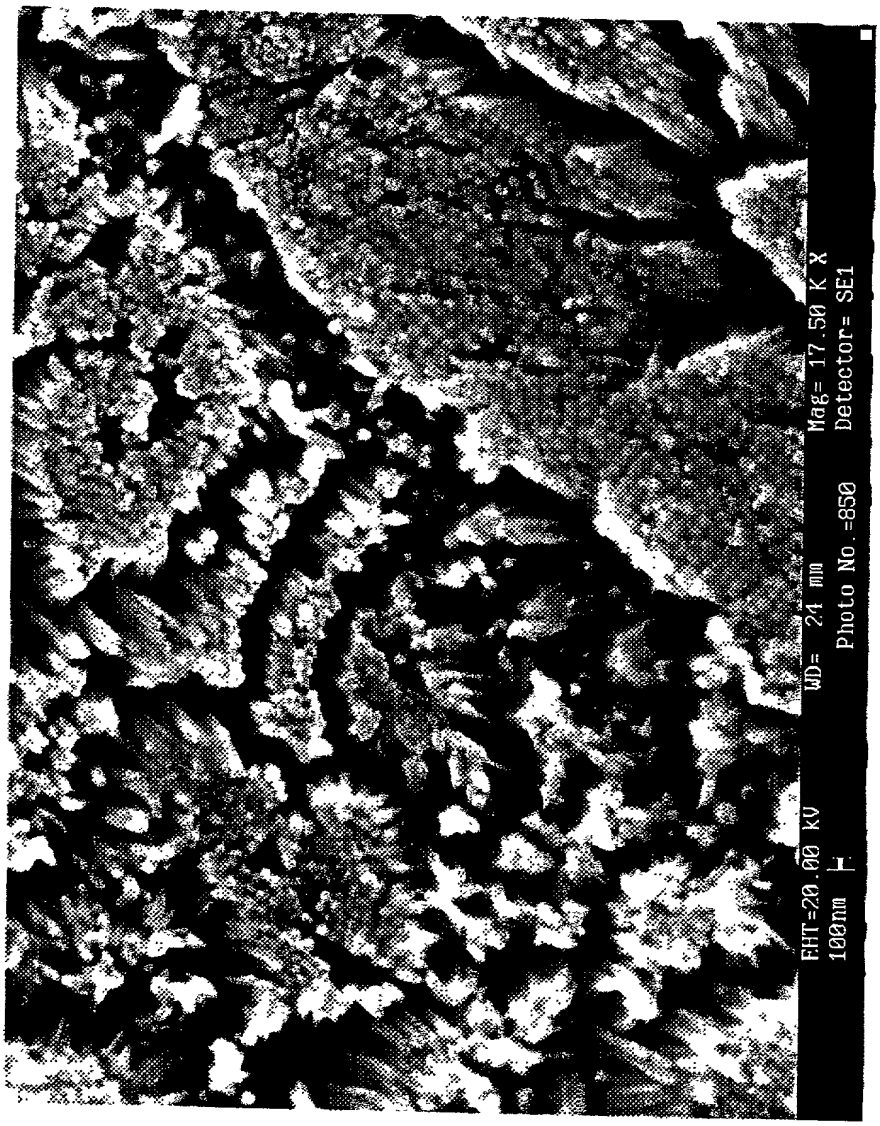
FIGURE 2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WD= 21 mm
Mag= 17.50 K X
100nm
Photo No.=850
Detector= SE1

FIGURE 3

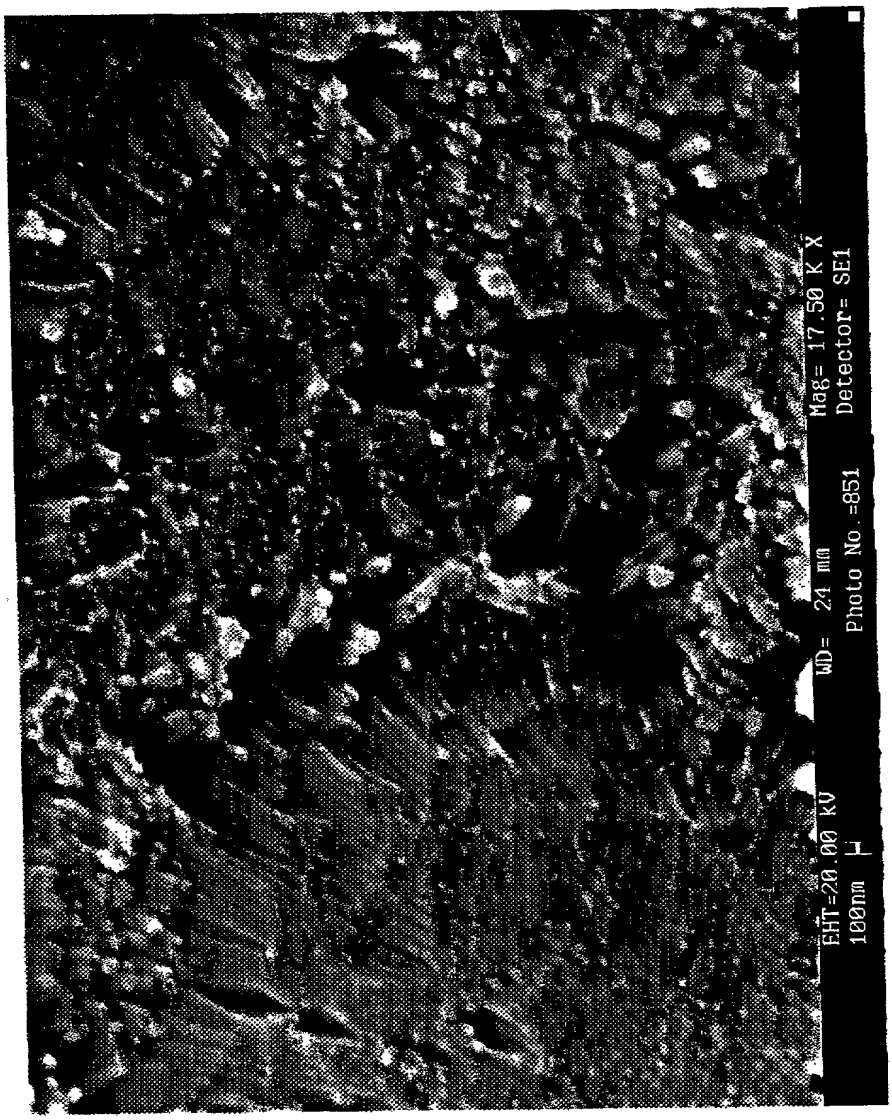
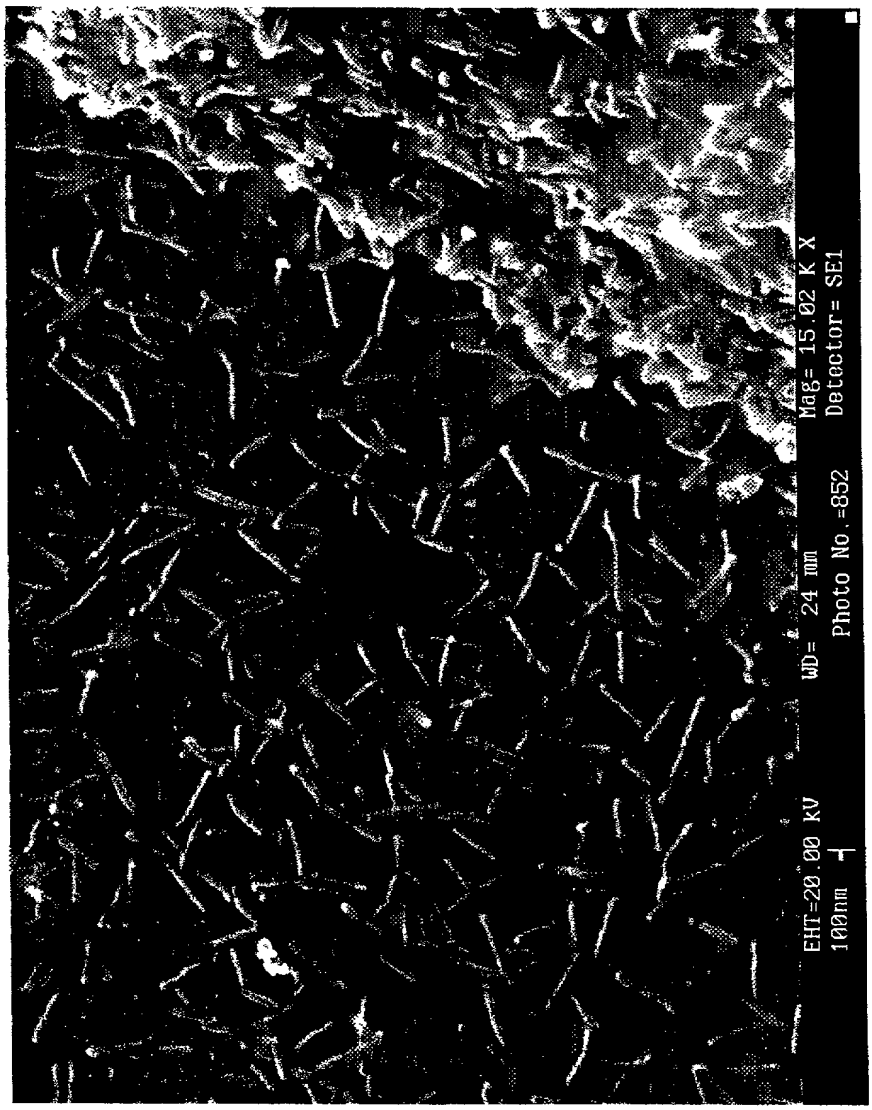

EHT=20.00 kV
WD= 24 mm
Mag= 17.50 K X
100nm
Photo No.=851
Detector= SE1

FIGURE 4


Mag= 15.02 K X
Detector= SE1
WD= 22 mm
Photo No.=854
EHT=20.00 kV
100nm H

FIGURE 4a

FIGURE 5


EHT=20.00 kV
WD= 24 mm
Mag= 15.02 K X
100nm
Photo No.=852
Detector= SE1

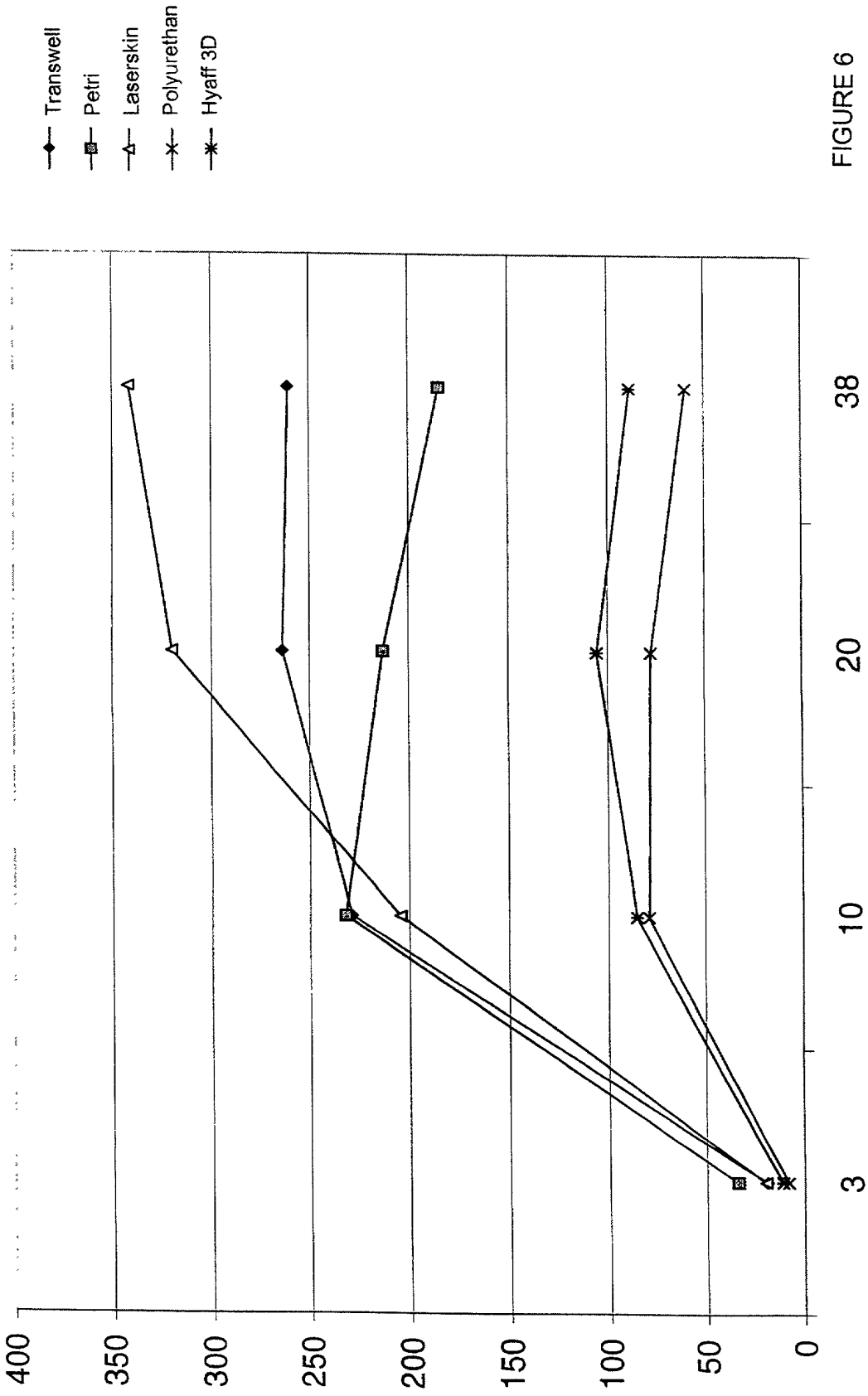

Transwell
Petri
Laserskin
Polyurethan
Hyaff 3D
400
350
300
250
200
150
100
50
0
3
10
20
38

FIGURE 6

UNITED STATES

PATENT APPLICATION DECLARATION AND POWER OF ATTORNEY - ORIGINAL APPLICATION	ATTORNEY'S DOCKET NO.

As a below named Inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name:
I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the invention entitled

TITLE OF VENTION

(1) BIOCOMPATIBLE AND BIODEGRADABLE COMPOSITIONS CONTAINING HYALURONIC ACID AND THE DERIVATIVES THEREOF FOR THE TREATMENT OF ULCERS IN THE DIGESTIVE APPARATUS

the specification of which

(2) ☐ is attached hereto.

2) CHECK PROPRIATE BOX

☐ was filed on _____ as Application No. _____

and was amended on _____ (if applicable).

☒ was filed as PCT international application number PCT/EP99/04604 on 2 July 1999

☐ and was amended under PCT Article 19 on _____ (if applicable)

OIPE FEB 21 2001 JC46 PATENT & TRADEMARK OFFICE

02-21-2001
U.S. Patent & TMOfc/TM Mail Rcpt Dt. #66

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.
I acknowledge my duty to disclose information of which I am aware which is material to the examination of this application under 37 CFR 1.56(a); the invention has not been patented or made the subject of a inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to this application; and
as to applications for patents or inventor's certificate on the invention filed in any country foreign to the United States prior to this application by me or my legal representatives or assigns,

(3) CHECK APPROPRIATE BOX

(3) ☐ no such applications have been filed, or

☐ such applications have been filed as follows:

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS PRIOR TO THIS APPLICATION				
Country	Application Number	Date of Filing (day, month, year)	Date of Issue (day, month, year)	Priority Claimed Under 35 USC 119
(4) ITALY	PD98A000168	6 July 1998		☒ Yes ☐ No
				☐ Yes ☐ No
ALL FOREIGN APPLICATIONS, IF ANY, FILED MORE THAN 12 MONTHS PRIOR TO THIS APPLICATION				
(4)				

(4) COMPLETE DATA INDICATED F APPLICABLE

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

(5) COMPLETE DATA INDICATED F APPLICABLE

(5) _____
(Application Ser. No.) (Filing date) (Status: patented, pending, abandoned)

(5) _____
(Application Ser. No.) (Filing date) (Status: patented, pending, abandoned)

1991PUS
USCR

Power of Attorney: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willfull false statements may jeopardize the validity of the application or any patent issued thereon.

(6) DETAILS REQUIRED FOR EACH INVENTOR

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Residence		**Citizenship**
Post Office Address		
Full Name of Sixth Joint Inventor, If Any	**Inventor's Signature**	**Date**
Residence		**Citizenship**
Post Office Address		

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